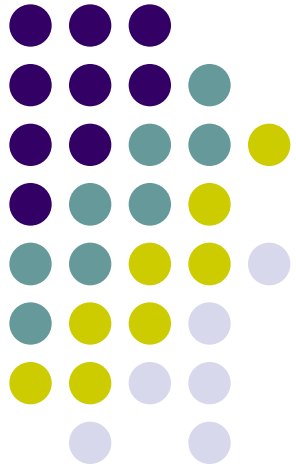
The Latest Trends In Tuberculosis Testing

Overview Of Specialized Lab Tests



Dr.Shamma Shetye

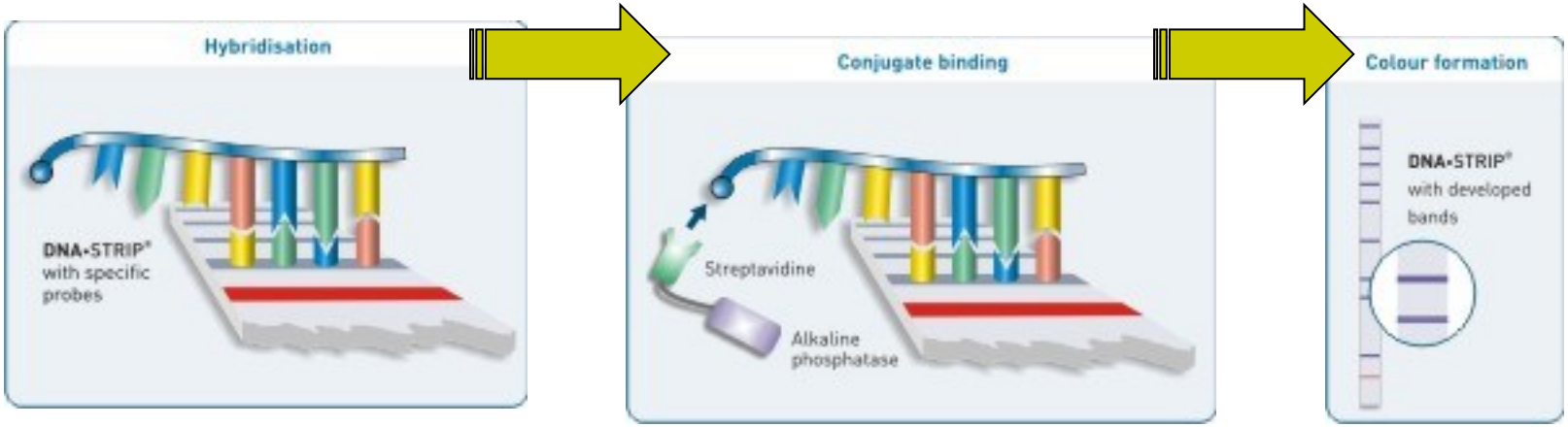
Metropolis Healthcare, Mumbai

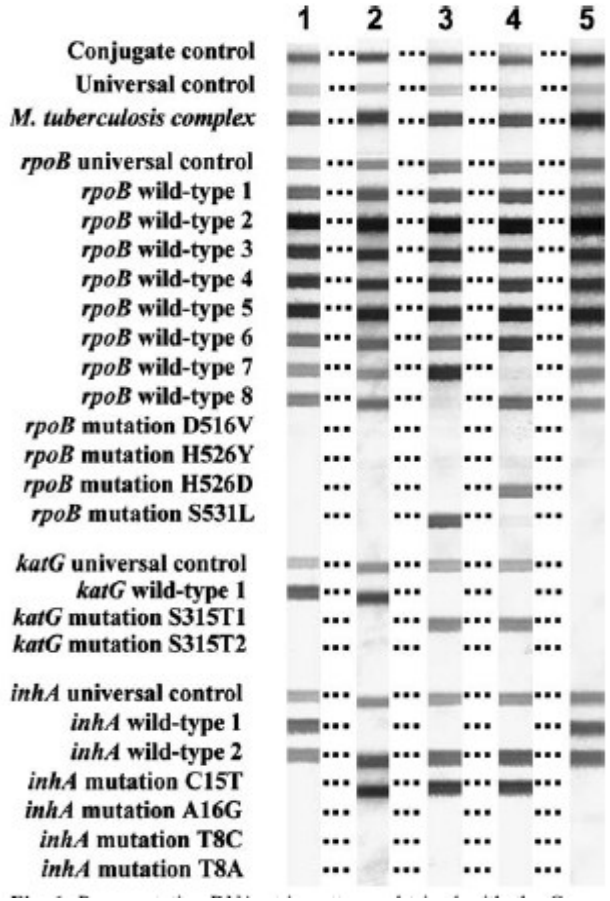
Genotype® MTBDR*plus* assay

- The Genotype® MTBDR*plus* assay) was performed according to the manufacturer's instructions.

- The Genotype MTBDRplus assay (Hain Lifescience GmbH, Nehren,Germany) :Molecular line probe assay to detect gene mutations that signal drug resistance.

- DNA strip technology ---Three steps: DNA extraction from cultured material (Solid/Liquid medium) or clinical specimens, multiplex polymerase chain reaction (PCR) amplification, and reverse hybridization.





- Each strip consists of 27 reaction zones (bands), including six controls (conjugate, amplification, *M. tuberculosis* complex, *rpoB*, *katG* and *inhA* controls), eight *rpoB* wild-type (WT) and four mutant (MUT) probes, one *katG* wild-type and 2 mutant probes, and two *inhA* wild-type(WT) and four mutant (MUT)probes.

Result interpretation:

- Presence of mutation (MUT) and absence of wild type (WT) indicates resistance.

Result

- Out of 78 samples tested, 15 were RIF resistant, 19 were INH resistant and 11 were Multi-drug resistant by conventional method (Versa TREK).
- The genotype MTBDR plus assay was able to detect RIF resistance accurately in 100% (15/15) and INH resistance in 89.4% (17/19).
- Of the 11 MDR isolates, clear cut recognition of RIF and INH resistance was found in 90% (10/11) of the samples.
- The overall concordance of MTBDR plus and the Versa TREK system for detecting RIF and INH susceptibility was 98.3% (76/78) and 94.8% (74/78) respectively.

Discussion

- **Resistance to INH is conferred by mutations:**

  Kat peroxidase gene (kat g) 50-90%

  Enocyl-acyl carrier protein (inhA) 15-35%

  Ahp oxy R intergenic region 10-15%

- **Resistance to RIF :**

  RNA polymerase B subunit gene

  rpoB mutations 96%

Mutations

- rpoB S531L most common mutation (RIF)
- kat G S315T1 ---(INH)
- inh A C15T-------(INH low level resistance).

EXPERT REVIEWS

Rapid diagnosis of drug-resistant TB using line probe assays: from evidence to policy

Expert Rev. Resp. Med. 2(5), 583–588 (2008)

Daphne I Ling, Alice A Zwerling and Madhukar Pai†

†Author for correspondence
Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Respiratory Epidemiology and

Growing concerns about the spread of multidrug-resistant tuberculosis (MDR-TB) and the emergence of extensively drug-resistant TB have triggered substantial interest in the development and application of rapid tests for the detection of drug-resistant TB. Molecular assays to detect gene mutations that signal drug resistance are widely recognized as being most suited for rapid diagnosis. Among molecular assays, line probe assays have shown great promise. Currently, two line probe assays are commercially available: the INNO-LiPA® Rif. TB kit (Innogenetics NV, Gent, Belgium) and the GenoType® MTBDR*plus* assay (Hain Lifescience GmbH, Nehren, Germany). Evidence from a systematic review suggests that INNO-LiPA is a highly sensitive and specific test

Table 1. Findings from systematic reviews of commercial line probe assays for the diagnosis of drug-resistant tuberculosis.

Focus of the systematic review	Tests evaluated	Studies in the meta-analysis (n)	Specimens in the meta-analysis (n)	Studies that used the assay directly on clinical specimens (n)	Principal findings and conclusions	Ref.
Rapid detection of rifampicin resistance	INNO-LiPA® Rif. TB assay	15	3349	4	12 out of 14 studies applying LiPA to isolates had a sensitivity greater than 95%, and 12 out of 14 had a specificity of 100%; the four studies that used clinical specimens had 100% specificity, and sensitivity ranged from 80 to 100%; overall, the LiPA assay was highly accurate when used on isolates; more evidence is needed on its accuracy when used directly on clinical specimens	[8]
Rapid detection of rifampicin and isoniazid resistance	GenoType® MTBDR assay and GenoType MTBDR*plus* assay	14 for rifampicin 15 for isoniazid	1738	9 for rifampicin 10 for isoniazid	The pooled sensitivity (98.1%; 95% CI: 95.9–99.1) and specificity (98.7%; 95% CI 97.3–99.4) estimates for rifampicin resistance were very high and consistent across all groups, assay versions and specimen types; the accuracy for isoniazid was variable, with sensitivity lower (84.3%; 95% CI: 76.6–89.8) and more inconsistent than specificity (99.5%; 95% CI: 97.5–99.9); overall, GenoType MDTBR assays had excellent accuracy for rifampicin resistance, even when used on clinical specimens; while specificity was excellent for isoniazid, sensitivity estimates were modest and variable	[9]

CI: Confidence Interval.

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ARTICLE

Multidrug-resistant tuberculosis: rapid detection of resistance to rifampin and high or low levels of isoniazid in clinical specimens and isolates

R. Vijdea · M. Stegger · A. Sosnovskaja · Å. B. Andersen · V. Ø. Thomsen · D. Bang

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Abstract The aim of the present study was to evaluate a new improved multiplex polymerase chain reaction (PCR) hybridisation assay to detect multidrug-resistant tuberculosis. The assay, developed to detect rifampin (*rpoB*) and isoniazid (*katG*) gene mutations causing *Mycobacterium tuberculosis* resistance, was recently extended to include *inhA* gene mutations that code for low-level isoniazid resistance. Interpretable results were obtained in 115 isolates and in all smear-positive clinical specimens. Rifampin resistance was correctly identified in all specimens and in 20 of 21 (95%) multidrug-resistant isolates compared to BACTEC 460TB. Isoniazid resistance correlated in 18 of 22 (82%) specimens, in 31 of 31 (100%) high-level and 24 of 28 (86%) low-level isoniazid-resistant isolates. The assay was rapid, easy to perform and directly applicable in smear-positive specimens. We predict that the assay may be a useful tool to combat and prevent new cases of multi- and extensively drug-resistant tuberculosis.

R. Vijdea · M. Stegger · V. Ø. Thomsen · D. Bang (✉)
International Reference Laboratory of Mycobacteriology and National Centre for Antimicrobials and Infection Control,
Statens Serum Institut,
Artillerivej 5,
2300 Copenhagen, Denmark
e-mail: DVB@SSI.DK

A. Sosnovskaja
National Tuberculosis Reference Laboratory,
National Tuberculosis and Infectious Disease University Hospital,
Antakalnio 77,
Vilnius 2040, Lithuania

Å. B. Andersen
Department of Infectious Diseases, Rigshospitalet,
Blegdamsvej 9,
2100 Copenhagen, Denmark

Introduction

Multidrug-resistant tuberculosis (MDR-TB) is defined as disease caused by *Mycobacterium tuberculosis* resistant to at least rifampin (RIF) and isoniazid (INH), the two most important first-line anti-TB drugs. MDR-TB represents a serious problem for clinical management, and has become an important public health issue. The rapid detection of drug resistance permits the establishment of an effective treatment regimen, minimises the risk of further resistance development and limits the spread of drug-resistant *M. tuberculosis*.

Over 400,000 MDR-TB cases emerge every year, 50% amongst new TB cases and 50% in previously treated TB patients [1]. Approximately 5–7% of these cases are expected to have extensively drug-resistant TB (XDR-TB) [2]. MDR-TB and XDR-TB are associated with an extremely high mortality, especially in the human immunodeficiency virus (HIV) coinfected [3, 4]. Therefore, rapid methods to determine drug resistance are urgently in demand.

Resistance to INH is conferred by mutations within the catalase-peroxidase enzyme gene (*katG* in 50–95% of resistant strains), the enoyl-acyl carrier protein reductase gene (*inhA* in 15–35% of resistant strains) and in the *ahpC–oxyR* intergenic region (10–15% of resistant strains) [5–9]. Resistance to RIF is predominantly linked to mutations within the RNA polymerase, B-subunit gene (*rpoB* in approximately 96% of all RIF-resistant stains tested) [10].

Recently, a combined multiplex polymerase chain reaction (PCR) and DNA strip hybridisation assay, the GenoType MTBDR*plus* (GT*plus*), was further developed from the original GenoType MTBDR (GT) version (Hain Lifescience, Nehren, Germany). The technique combines a multiplex PCR followed by the hybridisation to specific membrane-bound probes for the identification of either wild-type (WT) or specific mutations. In addition to the detection of mutations in

Rapid Molecular Screening for Multidrug-Resistant Tuberculosis in a High-Volume Public Health Laboratory in South Africa

Marinus Barnard[1], Heidi Albert[2], Gerrit Coetzee[3], Richard O'Brien[2], and Marlein E. Bosman[1]

[1]National Health Laboratory Services (NHLS), Greenpoint, Cape Town, South Africa; [2]Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland; and [3]National TB Reference Laboratory, NHLS, Sandringham, Johannesburg, South Africa

Rationale: The dual challenges to tuberculosis (TB) control of HIV infection and multidrug resistance are particularly pressing in South Africa. Conventional methods for detecting *Mycobacterium tuberculosis* drug resistance take weeks to months to produce results. Rapid molecular testing for drug resistance is available but has not been implemented in high-TB-burden settings.

Objectives: To assess the performance and feasibility of implementation of a commercially available molecular line-probe assay for rapid detection of rifampicin and isoniazid resistance.

Methods: We performed the assay directly on 536 consecutive smear-positive sputum specimens from patients at increased risk of multidrug-resistant (MDR) TB in a busy routine diagnostic laboratory in Cape Town, South Africa. Results were compared with conventional liquid culture and drug susceptibility testing on solid medium.

Measurements and Main Results: Overall, 97% of smear-positive specimens gave interpretable results within 1–2 days using the molecular assay. Sensitivity, specificity, and positive and negative predictive values were 98.9, 99.4, 97.9, and 99.7%, respectively, for detection of rifampicin resistance; 94.2, 99.7, 99.1, and 97.9%, respectively, for detection of isoniazid resistance; and 98.8, 100, 100, and 99.7%, respectively, for detection of multidrug resistance compared with conventional results. The assay also performed well on specimens that were contaminated on conventional culture and on smear-negative, culture-positive specimens.

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Molecular assays for diagnosis of drug-resistant tuberculosis (TB) are available but not widely used. There is no information on their performance in multidrug-resistant (MDR) TB screening in high-burden settings.

What This Study Adds to the Field

Molecular assays for MDR TB diagnosis from sputum specimens can be implemented in high-burden settings. The high accuracy, large reduction in reporting time, and high-volume capacity suggest the assay may revolutionize MDR TB diagnosis.

in KwaZulu-Natal, South Africa, 52 of 53 patients died, with a median survival time of 16 days from the date of diagnosis (6).

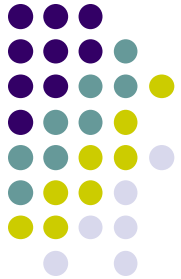
The diagnosis of MDR and XDR TB is based on mycobacterial culture and drug susceptibility testing (DST) on liquid or

Denmark study

- 115 isolates..
- RIF resistance 98%
- INH (kat G) 100%
- INH(inh A) 86%
- rpo B S531 L
- kat G S315T1

S.Africa study

- 531 isolates
- RIF resistance 98.9%
- INH resistance 94.2%
- MDR 98.8%
- Specificity for both drugs was 99.7% and 100% for MDR.



- Smear positive specimens or culture isolate
- Technical expertise
- Stringent quality control

- Silent mutations
- Heteroresistance